

**Reinstatement of *Phascolosoma* (*Phascolosoma*) *varians* Keferstein, 1865 (Sipuncula:
Phascolosomatidae) based on morphological and molecular data**

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Abstract

Phascolosoma (*P.*) *varians*, a species from the Greater Caribbean, was considered as a synonym of *Phascolosoma* (*P.*) *nigrescens*, a species from Fiji because both species were found morphologically indistinguishable. After the assignation of the synonymy, no detailed analysis of morphological characters nor genetic analysis have corroborated the supposed widespread distribution of *Phascolosoma* (*P.*) *nigrescens*.

In this study, *Phascolosoma* (*P.*) *varians* is redescribed, and differences concerning its supposed senior synonym were found. Both species differ mainly by the shape of the hooks, attachment of nephridia to the body wall, and the contractile vessel. Also, a high genetic divergence in nucleotide sequences of COI was found between the species, supporting the morphological data. Herein, the synonymy of *Phascolosoma* (*P.*) *varians* with *Phascolosoma* (*P.*) *nigrescens* is rejected due to the morphological and molecular differences; thus, the widespread distribution of *Phascolosoma* (*P.*) *nigrescens* is still considered questionable.

Introduction

The phylum Sipuncula comprised of 320 species (Stephen & Edmonds, 1972), but after Edward and Norma Cutler revisions, the number was reduced to 149 valid species (Cutler, 1994). Since 1994, 13 new species have been described (Kawauchi & Rice, 2009; Hylleberg, 2013; Saiz *et al.*, 2015; Silva-Morales *et al.*, 2019). Particularly, in the Greater Caribbean region, 38 valid species have been recorded, from which 40% corresponds to species with type localities outside Great Caribbean, with seven belonging to Phascolosomatidae (Quiroz-Ruiz & Londoño-Mesa, 2015).

The reduction in the number of species produced an extensive list of synonyms, which triggered subsequent incorrect identifications. This approach was supported by the idea of a wide distribution of sipunculan species, based on the supposed high dispersal capability of some species with pelagosphere larva, which have been evidenced that can remain in the water column for up to six months under laboratory conditions (Rice, 1976). However, some recent works, based on molecular data, clarified some taxonomical problems at the species level, showing that small morphological differences correspond to different species, rejecting the supposed wide distribution of some species (Staton & Rice, 1999; Kawauchi & Giribet, 2010; Schulze *et al.*, 2012; Kawauchi & Giribet, 2014; Johnson *et al.*, 2016; Silva-Morales *et al.*, 2019). For that reason, Kawauchi, Sharma & Giribet (2012) proposed to clarify the taxonomic status of each species through a meticulous case-by-case analysis, integrating molecular data, and considering that some species names included as synonyms, deserve to be restored.

Keferstein (1865) described *Phascolosoma* (*Phascolosoma*) *varians* from St. Thomas, West Indies, and *P.* (*P.*) *nigrescens* from Fiji. Cutler & Cutler (1990) reviewed the subgenus *Phascolosoma* (*Phascolosoma*), and they included 15 species described all around the world as synonyms of *P.* (*P.*) *nigrescens*. The identification key by Cutler (1994) indicates that the diagnostic character of *P.* (*P.*) *nigrescens* is: Clear streak of hook with swelling in middle of vertical and horizontal portion. *Phascolosoma* (*P.*) *varians*, was one of the species included as junior synonymy of *P.* (*P.*) *nigrescens*.

Comentado [UdMO1]: Genetic is not the same as molecular

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Comentado [UdMO2]: Are explained?

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Comentado [UdMO3]: Was not Cutler & Culter, 1990? The idea says "... after Edward and Norma Cutler revisions..."

Comentado [ML4]: Or since 2009? Why is since 1994 without the reference from 1994?

Comentado [ML5]: Greater or Great? Why not Grand?

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Comentado [ML7]: Is this correct following the author instructions? Three last names? From how many you have to put *et al.*? check

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Con formato: Fuente: Sin Cursiva

90 Cutler (1994) considered the distribution of this species as very widespread
 91 circumtropical, generally, between 30° N and 30° S, in shallow waters of the Indian, Pacific, and
 92 Atlantic oceans. In the Greater Caribbean, *Phascolosoma* (*P.*) *nigrescens* has been reported by
 93 Cutler & Schulze (2004) from Barbados, Schulze & Rice (2004) from Belize, Schulze (2005)
 94 from Panama, and Frontana-Urbe *et al.* (2018) from the Mexican Caribbean.

95 Herein, a detailed redescription of *P. (P.) varians* based upon topotypic specimens and
 96 additional material from the Caribbean region is provided. Furthermore, *P. (P.) varians* is re-
 97 established due to the morphological and molecular differences concerning its supposed senior
 98 synonym, *P. (P.) nigrescens*.
 99

100 Materials & Methods

101 Specimens from the collections of the Marine Invertebrate Museum (UMML), Rosenstiel School
 102 of Marine and Atmospheric Science, University of Miami; Invertebrate Collections of the
 103 Florida Museum of Natural History (UF-FLMNH), University of Florida, and the Reference
 104 Collection of Benthos (ECOSUR) of El Colegio de la Frontera Sur, Chetumal, Mexico were
 105 reviewed.

106 The redescription of the species was mainly based on a topotypic specimen, but
 107 variations were complemented with additional materials from the Caribbean region.
 108 Standardized descriptions included external and internal anatomy; the descriptions of hooks and
 109 papillae followed the terminology described by Cutler (1994). To measure the angle between the
 110 primary tooth and the hook, a line X was drawn perpendicular to the base through the most
 111 anterior part of the concave side, and a line Y was drawn from the tip until intersect X in the
 112 middle of the point (see Cutler 1994:161-162, fig. 44A).

113 Hooks and papillae were extracted with fine forceps to study them under an optical
 114 microscope. Hooks were removed from three different regions (anterior, median and posterior)
 115 of the ringed area. Papillae were described from three different regions (anterior, middle and
 116 posterior) of the trunk, and also from the base of the introvert. Furthermore, these structures were
 117 examined using SEM to achieve a more detailed examination. Therefore, the complete introvert
 118 was dehydrated in a series of different concentration of hexamethyldisilazane (HMDS), and air-
 119 dried. Then, it was mounted on an aluminum stub and coated with gold for observation using a
 120 JEOL JSM-6010Plus-LA scanning electron microscope at the Scanning Electron Microscopy
 121 Laboratory (LMEB), ECOSUR-Chetumal.

122 A series of digital photographs of some internal and external features were taken using a
 123 Canon X6 digital camera mounted on a microscope. All images were stacked using
 124 HeliconFocus v6.7.1 (HeliconSoft 2007) to improve field depth.

125 For molecular analyses, nine sequences of specimens identified as *Phascolosoma* (*P.*)
 126 *nigrescens* were retrieved from GenBank, three of them from the Western Atlantic (DQ300139,
 127 DQ300142, AY161122), three from Australia (DQ300143, JN865121, JN865122), one from
 128 Israel (DQ300140), and another from South Africa (DQ300141). Also, a sequence of
 129 *Phascolosoma* (*P.*) *granulatum* Leuckart, 1828 (DQ300138) and two sequences of

Comentado [ML10]: Very? How much? Author must avoid to use this kind of words that implies an imprecise qualification.

Eliminado: ;

Comentado [ML11]: A redescription must be detailed.

Comentado [ML12]: But topotypic material is also from the Caribbean. Re-writer sentence.

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Comentado [ML13]: From other Caribbean localities

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Eliminado: is

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Comentado [ML15]: Middle point?

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Comentado [ML17]: Compound

Comentado [ML18]: This terminology apply for the body. The correct terminology for an "apendix", like the introvert, is proximal, middle and distal. Distal is near the mouth.

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Comentado [ML19]: DNA or RNA?

Comentado [ML20]: Could be more precise at least on this, because the Caribbean also is in the Western Atlantic. So, if you are trying to solve the problem on distribution, this could be important.

140 *Phascolosoma* (*P.*) *agassizii* Keferstein, 1866 (JQ904338, JQ904337) were included for
141 comparison.

142 All sequences were aligned using ClustalW method; the selection of the best model of
143 substitution was determined according to the lowest Bayesian Information Criterion scores
144 (BIC). As a result, Tamura 3-parameter (Tamura, 1992), using a discrete Gamma distribution
145 (+G) with five categories and by assuming that a certain fraction of sites is evolutionarily
146 invariable (+I) was used as model to construct a tree using maximum likelihood analysis. Also,
147 the Kimura 2-parameter model (Kimura, 1980) was used to estimate the average evolutionary
148 divergence over sequence pairs within and between species. All analyses were carried out with
149 Mega 7 (Kumar, Stecher & Tamura, 2015).

151 Results

153 Systematics

155 Family Phascolosomatidae Stephen & Edmonds, 1972

157 *Phascolosoma* Leuckart, 1828

159 *Phascolosoma* (*Phascolosoma*) Leuckart, 1828

161 **Type species.** *Phascolosoma granulatum* Leuckart, 1828

163 **Diagnosis.** Body wall muscles separated into distinct bands. Spindle muscle attached posteriorly;
164 introvert hooks without accessory spinelets (after Cutler 1994).

166 *Phascolosoma* (*Phascolosoma*) *varians* Keferstein, 1865 reinstated

168 *Phascolosoma varians* Keferstein 1865: 424-426, pl. 32, Fig. 22. de Quatrefages 1865: 623.

169 Wesenberg-Lund 1954: 7-8.

170 *Physcosoma varians* ten Broeke 1925: 85.

171 *Phymosoma varians* Selenka 1883: 69-70, pl. 9, figs. 124-127. Shipley 1890: 1-24, fig. pls. 1-4,
172 figs. 1-32.

173 *Phascolosoma* (*Phascolosoma*) *varians* Stephen & Edmonds 1972: 327-328, fig. 39I.

174 *Phascolosoma nigrescens* Cutler 1994 (*partim*): 179-181; Cutler & Schulze 2004: 226; Rice &
175 Macintyre 1982: 314; Schulze 2005: 526; Frontana-Urbe *et al.* 2018: 174, fig. 5a-b; (non
176 Keferstein, 1865).

178 Material examined

Comentado [ML21]: As sister/out groups? Use precise terminology

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Comentado [ML22]: Thus

Comentado [ML23]: grammar

Comentado [ML24]: Check this... three authors? Et al.?

Comentado [ML25]: Not in references

Comentado [ML26]: Because it is a reference, you must use coma between the name and the reference. Otherwise, it could be understood as the author of the species, which is wrong.

Comentado [ML27]: Check if "de" must be included.

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Comentado [ML28]: Is there any order in the localities? I recomend to use a geographical order from North to South and West to East. Or alfabetical? Any suggested order is accpetable, but use any. Even, inside each country.

184 **Florida, USA**. UMML 26.5, 2 specimens, Bear Cut, Key Biscayne, 25°43'54.33"N,
 185 80°09'26.16"W, May 7, 1961, coll. P. Robertson. UMML s/n, 1 specimen. Margot Fish Shoal,
 186 Dade Co, Apr 5, 1966, coll. G. Hendrix, bored in coral rubble. UF 292, 1 specimen, N of St.
 187 Petersburg, 28°27'33.12"N, 84°16'18.48"W, 30 m, Mar 13, 2011, colls. G. Paulay, N. Evans, F.
 188 Michonneau, C. Thacker, R. Williams, A. Baeza. UF 293, 1 specimen, N of St. Petersburg,
 189 28°27'33.12"N, 84°16'18.48"W, hard ground with sponges, 30 m, Mar 13, 2011, coll. G. Paulay.
 190 UF 324, 1 specimen, St. Petersburg, 28°33'24.12"N, 84°16'28.20"W, hard bottom, sponge reef,
 191 27 m, May 24, 2012, coll. J. Slapcinsky. UF 325, 1 specimen, St. Petersburg, 28°39'03.96"N,
 192 84°23'03.84"W, 26-30 m, hard bottom, sponge reef, May 25, 2012, colls. G. Paulay, N. Evans, F.
 193 Michonneau. **Mexico, Mexican Caribbean. Isla Contoy**. ECOSUR-S62, 3 specimens, Morro
 194 Norte, 21°28'32.79"N, 86°47'30.13"W, coralline rock, 2.5 m, Feb 26, 2008, colls. S. Salazar-
 195 Vallejo, L. Carrera-Parra. ECOSUR-S63, 2 specimens. Punta Sur, 21°27'37.10"N,
 196 86°47'04.60"W, coralline rock, 1.5 m, Mar 2, 2001, colls. S. Salazar-Vallejo, L. Carrera-Parra.
 197 ECOSUR-S69, 1 specimen, Punta Sur, 21°27'37.10"N, 86°47'04.60"W, coralline rock, 1.5 m,
 198 Feb 28, 2001, colls. S. Salazar-Vallejo, L. Carrera-Parra. ECOSUR-S70, 1 specimen, Ixlache
 199 reef, 21°26'02.52"N, 86°46'56.16"W, coralline rock, 2 m, Feb 25, 2008, colls. S. Salazar-Vallejo,
 200 L. Carrera-Parra. **Cancún, Punta Nizuc**. ECOSUR-S87, 1 specimen, 21°01'41.92"N,
 201 86°46'45.72"W, coralline rock, 2.6 m, Aug 31, 1997, colls. S. Salazar-Vallejo, L. Carrera-Parra,
 202 M. Ruiz-Zárate. ECOSUR-S88, 2 specimens, 21°01'17.06"N, 86°46'45.95"W, coralline rock, 4
 203 m, Sep 1, 1997, colls. S. Salazar-Vallejo, L. Carrera-Parra, M. Ruiz-Zárate. ECOSUR-S89, 3
 204 specimens, 21°01'17.06"N, 86°46'45.95"W, coralline rock, 4 m, Sep 1, 1997, colls. S. Salazar-
 205 Vallejo, L. Carrera-Parra, M. Ruiz-Zárate. ECOSUR-S90, 2 specimens, 21°01'17.06"N,
 206 86°46'45.95"W, coralline rock, 4 m, Sep 1, 1997, colls. S. Salazar-Vallejo, L. Carrera-Parra, M.
 207 Ruiz-Zárate. **Playa del Carmen**. ECOSUR-S86, 4 specimens, Navega pier, 20°37'12.07"N,
 208 87°04'26.63"W, fouling, 1 m, Aug 23, 2003, coll. M. Tovar-Hernández. **Cozumel**. ECOSUR-
 209 S64, 14 specimens, Playa Azul, 20°32'51.98"N, 86°55'46.45"W, coralline rock, 1 m, Mar 25,
 210 2001, colls. S. Salazar-Vallejo, L. Carrera-Parra. ECOSUR-S65, 1 specimen, in front to
 211 SEDENA, 20°31'00.61"N, 86°56'45.52"W, coralline rock, 1.5 m, Mar 24, 2001, colls. S.
 212 Salazar-Vallejo, M. Londoño-Mesa. **Tulum**. ECOSUR-S66, 3 specimens, Playa Aventuras,
 213 20°21'47.20"N, 87°19'53.10"W, coralline rock, 1.5 m, Feb 28, 1999, colls. S. Salazar-Vallejo, J.
 214 Bastida-Zavala. ECOSUR-S91, 1 specimen, Playa Aventuras, 20°21'47.20"N, 87°19'53.10"W,
 215 coralline rock, 1.5 m, Feb 18, 2001, colls. S. Salazar-Vallejo, L. Carrera-Parra. ECOSUR-S92, 4
 216 specimens, Ana y José beach, 20°09'24.22"N, 87°27'13.74"W, coralline rock, 1 m, Feb 11, 2001,
 217 colls. S. Salazar-Vallejo, J. Bastida-Zavala, J. Ruiz-Ramírez. ECOSUR-S96, 3 specimens, Punta
 218 Piedra, 20°10'38.94"N, 87°26'42.28"W, coralline rock, 1.5 m, Feb 11, 2001, colls. S. Salazar-
 219 Vallejo, L. Carrera-Parra, M. Tovar-Hernández. **Mahahual**. ECOSUR-S67, 1 specimen, 25 m
 220 off coast, 18°43'27.09"N, 87°42'3.64"W, reef lagoon, rocky substrate with sediment, 0.75 m,
 221 Oct 1, 1996, colls. S. Salazar-Vallejo, L. Carrera-Parra. ECOSUR-S71, 1 specimen, in sponge,
 222 Jul 21, 1998. ECOSUR-S72, 2 specimens, 50 m off coast, 18°43'38.68"N, 87°41'56.81"W,
 223 coralline rock, 2 m, Mar 4, 1998, colls. S. Salazar-Vallejo, L. Carrera-Parra. ECOSUR-S73, 6

Comentado [ML29]: If you say Mexico, Mexican Caribbean, you have to say here, USA, Florida. Check this in order to have all standardized.

Comentado [ML30]: It could be reduced as (2), indicating in methods that this means number of specimens. Thus, you could save space in the paragraph deleting all the "specimens".

Comentado [ML31]: You could also reduce this as 05.04.1966 (DD.MM.YYYY). You could mention this in methods.

Comentado [ML32]: Recommend to avoid using italics. Only use it for genus and species names.

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Comentado [ML33]: If you use accent here, you have to use it in México, Panamá, etc... Standardize.

specimens, reef lagoon near to back reef, 18°42'34.01"N, 87°42'31.22"W, coralline rock, 1.5 m, Jan 9, 2001, colls. P. Salazar-Silva, J. Bastida-Zavala, M. Tovar-Hernández, S. Salazar-Vallejo, L. Carrera-Parra. ECOSUR-S74, 1 specimen, fore reef, 18°42'43.32"N, 87°42'22.51"W, coralline rock, 15 m, Jun 6, 1998, coll. M. Ruiz-Zárate. ECOSUR-S75, 2 specimens, reef lagoon, 18°42'36.23"N, 87°42'31.60"W, coralline rock, 1.5 m, Dec 1, 2000, colls. S. Salazar-Vallejo, L. Carrera-Parra. ECOSUR-S76, 2 specimens, reef crest, 18°43'06.17"N, 87°42'14.17"W, coralline rock, 1 m, Jul 21, 1998, colls. S. Salazar-Vallejo, L. Carrera-Parra. ECOSUR-S77, 5 specimens, back reef, 18°42'31.30"N, 87°42'30.39"W, coralline rock, 2 m, Mar 22, 2000, colls. S. Salazar-Vallejo, L. Carrera-Parra. ECOSUR-S78, 1 specimen, reef lagoon, 18°42'36.17"N, 87°42'32.65"W, coralline rock, 1.5 m, Mar 21, 2000, colls. J. Bastida-Zavala, P. Salazar-Silva. ECOSUR-S79, 5 specimens, old wooden pier, 18°42'41.95"N, 87°42'35.98"W, fouling, 1 m, Feb 24, 2001, colls. P. Salazar-Silva, J. Bastida-Zavala, M. Tovar-Hernández, S. Salazar-Vallejo, L. Carrera-Parra, L. Harris. ECOSUR-S80, 8 specimens, old wooden pier, 18°42'41.95"N, 87°42'35.98"W, fouling, 1 m, Mar 18, 2001, colls. P. Salazar-Silva, J. Bastida-Zavala, M. Tovar-Hernández, S. Salazar-Vallejo, L. Carrera-Parra. ECOSUR-S81, 14 specimens, reef lagoon, 18°43'22.73"N, 87°42'03.08"W, coralline rock, 1.5 m, Mar 28, 2001, colls. L. Carrera-Parra, M. Londoño-Mesa, S. Salazar-Vallejo. ECOSUR-S82, 1 specimen, reef lagoon, 18°43'25.14"N, 87°42'01.75"W, coralline rock, 1.5, Jan 10, 2001, colls. L. Carrera-Parra, M. Londoño-Mesa, S. Salazar-Vallejo. ECOSUR-S83, 12 specimens, reef lagoon, 18°43'24.93"N, 87°42'02.95"W, coralline rock, 1 m, Jan 19, 2001, colls. P. Salazar-Silva, J. Bastida-Zavala, M. Tovar-Hernández, S. Salazar-Vallejo, L. Carrera-Parra. ECOSUR-S84, 2 specimens, reef lagoon, 18°43'24.93"N, 87°42'02.95"W, coralline rock, 1 m, Nov 30, 2000, colls. S. Salazar-Vallejo, L. Carrera-Parra. ECOSUR-S85, 13 specimens, reef lagoon, 18°43'21.01"N, 87°42'04.28"W, coralline rock, 1.5 m, Feb 24, 2001, colls. P. Salazar-Silva, J. Bastida-Zavala, M. Tovar-Hernández, S. Salazar-Vallejo, L. Carrera-Parra, L. Harris. ECOSUR-S93, 1 specimen, punta Rio Bermejo, 18°41'07.53"N, 87°43'05.83"W, coralline rock, 1 m, May 17 2002, colls. S. Salazar-Vallejo, M. García-Madrigal. ECOSUR-S94, 16 specimens, punta Rio Indio, 18°48'29.63"N, 87°39'58.82"W, coralline rock, 1.7 m, Mar 17, 2001, coll. L. Carrera-Parra. **Xahuayxol**, ECOSUR-S58, 8 specimens, reef lagoon, 18°30'11"N, 87°45'29"W, coralline rock, 2 m, Jun 1 1997, colls. S. Salazar-Vallejo, L. Carrera-Parra. ECOSUR-S59, 1 specimen, back reef, 18°30'11.45"N, 87°45'21.46"W, coralline rock, 12 m, Oct 30 1997, colls. R. Saenz-Morales, S. Salazar-Vallejo, L. Carrera-Parra. ECOSUR-S60, 4 specimens, reef lagoon, 120 m off coast, 18°30'41.34"N, 87°45'24.63"W, coralline rock, 1.5 m, Oct 31 1997, colls. S. Salazar-Vallejo, L. Carrera-Parra. ECOSUR-S61, 11 specimens, reef lagoon, 18°30'39.77"N, 87°45'24.80"W, coralline rock, 1.8 m, Jun 4, 1998, colls. S. Salazar-Vallejo, L. Carrera-Parra. ECOSUR-S68, 3 specimens, reef lagoon, 18°30'39.04"N, 87°45'25.09"W, coralline rock, 1.7 m, Sep 27, 1996, colls. S. Salazar-Vallejo, L. Carrera-Parra. ECOSUR-S95, 21 specimens, reef lagoon, 18°30'12.46"N, 87°45'29.79"W, coralline rock, 2 m, Jun 1, 1997, colls. S. Salazar-Vallejo, L. Carrera-Parra. ECOSUR-S97, 1 specimen, 100 m off coast, 18°30'41.43"N, 87°45'25.16"W, coralline rock, 2 m, Sep 28, 1996, coll. S. Salazar-Vallejo, L. Carrera-Parra. ECOSUR-S98, 1

specimen, reef lagoon, 18°30'12.46"N, 87°45'29.79"W, coralline rock, 2 m, Jun 1, 1997, colls. S. Salazar-Vallejo, L. Carrera-Parra. ECOSUR-S99, 6 specimens, reef lagoon, 18°30'13.71"N, 87°45'31.50"W, coralline rock, 1 m, Jun 2 1998, colls. S. Salazar-Vallejo, L. Carrera-Parra. ECOSUR-S100, 2 specimens, 100 m off coast, 18°30'15.08"N, 87°45'30.98"W, in sediment with *Thalassia testudinum*, 2 m, Sep 27, 1996, colls. S. Salazar-Vallejo, L. Carrera-Parra. **Xcalak**. ECOSUR-S101, 9 specimens, back reef, 18°15'50.40"N, 87°49'31.12"W, coralline rock, 1.7 m, Oct 25, 2002, colls. S. Salazar-Vallejo, L. Carrera-Parra, P. Salazar-Silva, M. Londoño-Mesa. **Nicaragua, Atlantic coast**. UMML 000, 1 specimen, R/V Pillsbury, Cruise 7101, sta. 1338, 12°52'00"N, 82°35'17.98"W, hillocks or low rounded mounds, 28 m, Jan 29, 1971, coll. G. Voss. **Providencia Island**. UMML 000, 1 specimen, R/V Pillsbury, Cruise 7101, sta. 1349, 13°33'00"N, 81°28'00"W, patch reef slightly southwest of Low Island surrounded by sand bottom, 3 m, Jan 30, 1971. **Dominican Republic**. UMML 000, 2 specimens. R/V Pillsbury, Cruise 7006, sta. 1272, off Cabo Rojo, 17°52'41.98"N, 71°41'12.01"W, 20-27 m, Jul 17, 1970, coll. J. Staiger. **Turks and Caicos, West Indies**. UMML 000. 1 specimen, Pillsbury Cruise 7106, sta. 1423, 21°40'59.98"N, 71°22'59.98"W, Jul 19, 1971. **Saint Martin, Îlet de L'embouchure**. UF 329, 1 specimen, 18°04'1.2"N, 63°00'39.6"W, reef flat lagoon with seagrass, 1 m, Apr 9, 2012, colls. G. Paulay, J. Slapcinsky, M. Bemis. UF 330, 5 specimens, 18°04'01.20"N, 63°00'43.20"W, reef flat lagoon with seagrass, 0-1 m, Apr 9, 2012, colls. G. Paulay, J. Slapcinsky, M. Bemis. UF 338. 1 specimen, 18°04'01.20"N, 63°00'43.20"W, reef, 1 m, Apr 17, 2012, coll. A. Anker. UF 340, 1 specimen, 18°04'01.20"N, 63°00'43.20"W, reef, 1 m, Apr 17, 2012, coll. A. Anker. UF 347, 1 specimen, 18°04'01.20"N, 63°00'43.20"W, reef, 0-1 m, Apr 17, 2012, colls. G. Paulay, J. Slapcinsky, A. Anker. UF 359, 3 specimens, steep rubbly reef slope, 2-10 m, Apr 22, 2012, colls. G. Paulay, F. Michonneau. **Mont Vernon**, UF 332, 1 specimen, Little Key, 18°06'00"N, 63°01'19.20"W, seagrass, sand, rocks, 0-2 m, Apr 11, 2012, coll. J. Slapcinsky. **Grande Caye**, UF 353, 1 specimen, 18°06'43.20"N, 63°01'08.40"W, coral rocks, 3 m, Apr 20, 2012, coll. A. Anker. **Rocher Créole**. UF 352. 1 specimen, 18°07'04.80"N, 63°03'21.60"W, reef, 9 m, Apr 18, 2012, colls. G. Paulay, J. Slapcinsky, M. Bemis. **NE side of St. Martin**, UF 336, 1 specimen, 18°07'48"N, 63°00'18"W, canyon with sponges, in algae, 13 m, Apr 11, 2012, coll. R. Renoux. **Île Tintamare**, UF 361, 3 specimens, Chicot, windward side of the island, 18°06'7.2"N, 62°58'58.80"W, reef, in rubble, 12-15 m, Apr 23, 2012, coll. G. Paulay. **Caye Verte**, UF 364, 1 specimen, 18°05'24"N, 63°00'43.2"W, reef with sand and grass, 7 m, Apr 25, 2012, coll. M. Bemis. **Panama. Bocas del Toro**. UF 126, 1 specimen, coll. C. Meyer. UF 485. 1 specimen, Punta Puebla, 9°22'01.20"N, 82°17'27.60"W, May 16, 2016, colls. M. Leray, F. Michonneau, R. Lasley. UF 486, 1 specimen, Punta Juan, 9°18'03.60"N, 82°17'38.40"W, May 16, 2016, coll. R. Lasley. UF 494, 1 specimen, Runway, 9°20'31.20"N, 82°15'36"W, May 23, 2016, colls. M. Leray, F. Michonneau, R. Lasley. UF 499. 1 specimen, Marina, 9°19'51.60"N, 82°14'38.40"W, May 28, 2016, colls. M. Leray, F. Michonneau, R. Lasley. UF 500, 2 specimens, Marina, 9°19'51.60"N, 82°14'38.40"W, May 28, 2016, colls. M. Leray, F. Michonneau, R. Lasley. UF 501, 1 specimen, Cayo Hermanas, 9°16'04.80"N, 82°21'07.20"W, May 30, 2016, colls. M. Leray, F. Michonneau, R. Lasley. UF 503, 1 specimen. Cayo Hermanas, 9°16'04.80"N,

Comentado [ML34]: Caribbean? Nicaraguan Caribbean? Use the same structure for all the localities.

Comentado [ML35]: This is in Colombia, no in Nicaragua.

Comentado [ML36]: You have to precise what is "West Indies". It is more than these islands. Maybe the use of Greater and Lesser Antilles is more adequate; even more correct, these two islands belongs to the Lucayan Archipelago. Check and correct.

Comentado [ML37]: I found that the acronym is UF-FLMNH. Check this.

Con formato

305 82°21'07.20"W, May 31, 2016, colls. M. Leray, F. Michonneau, R. Lasley. UF 541, 1 specimen,
306 Salt Creek, 9°16'48"N, 82°06'07.20"W, outer reef, *Agaricia* reef, 4-4.5 m, May 22, 2016, colls.
307 M. Leray, F. Michonneau, R. Lasley. UF 542, 1 specimen, Runway, 9°20'31.20"N, 82°15'36"W,
308 May 23, 2016, colls. M. Leray, F. Michonneau, R. Lasley, 4-4.5 m, lagoon fringing reef,
309 *Agaricia* reef.

311 **Redescription based on a male from St. Martin, *Mont Vernon*, West Indies (UF 332)**

313 **External anatomy.** Trunk 14 mm length (Fig. 1A); light brown with some darker
314 patches; papillae dark brown and light brown distributed dispersedly, most of them on dorsal
315 zone, scarce ventrally; papillae conglomerated in anal (Fig. 1B) and caudal (Fig. 1C) region, and
316 dispersed in middle region (Fig. 1D).

317 Introvert 10 mm in length (Fig. 1A), dark brown, color uniform ventrally and dorsally,
318 with some transversal bands light brown (Fig. 1E), posterior end of introvert with similar color
319 pattern as trunk, extensible collar small. Introvert papillae dark brown, smaller than those from
320 the trunk (Fig. 1F), located between rings of hooks, starting from the first ring (Fig. 2G). Nuchal
321 organ with wavy contour.

322 Hooks laterally compressed, arranged in sixty complete rings and some incomplete rings,
323 probably by abrasion. The rings are followed by a zone with scattered hooks. Most anterior
324 hooks (Fig. 2A, 2F, 2G) with distal tip (main tooth) at 90° angle with respect to the perpendicular
325 line of hook; length of distal tip not projecting beyond the base of the hook; secondary tooth
326 rounded; internal clear streak (apical canal) expanded near to midpoint of vertical and middle
327 horizontal portions of hook. Hooks of median region with larger secondary tooth (Fig. 2B), most
328 posterior hooks (Fig. 2C) with principal tooth smaller than its base, almost 25% less.

329 SEM allowed observing the growth of hooks and papillae (Fig. 2H); both are smaller in
330 the most anterior region and larger in the posterior end. The papillae of the introvert have three
331 stages of development. First stage, the smallest, spherical with a ring of short apical protrusions
332 (Fig. 2K) "dome shape" (fide Cutler 1994). Second stage, medium size, with appearance of two
333 units, one smallest with a ring of short apical protrusions, and broad base (Fig. 2J) "mammillate
334 form" (fide Cutler 1994). Third stage, largest, conical (Fig. 2I) "cone shape" (fide Cutler 1994).

335 **Internal anatomy** (Fig. 2D). A pair of nephridia occupying 80% of trunk length, open at
336 the same level as anus. Longitudinal musculature divided into 23 to anastomosed bands,
337 depending on where we count (anterior, middle, or posterior trunk). Two pairs of retractor
338 muscles; ventral pair attached to 8-9 bands starting from the third band after ventral nerve cord
339 (Fig. 2E), dorsal pair attached to 3-6 bands starting from the fifth band after ventral nerve cord.
340 Contractile vessel without swelling or villi. Spindle muscle attached posteriorly.

342 **Habitat.** Inside coralline rock and hard bottom, 1 to 30 m depth.

344 **Distribution.** Greater Caribbean from Florida to Venezuela.

Eliminado: -

Comentado [ML38]: "In length" or "long". And how much "in width" or "wide"?

Con formato: Fuente: Negrita

Comentado [ML39]: at random, randomly... etc.

Comentado [ML40]: in body is region, not zone

Eliminado: region

Eliminado: (Fig. 1C)

Eliminado: most

Eliminado: median

Eliminado: color

Comentado [ML41]: It is one specimen.. how many bands? This must be precise

Comentado [ML42]: I think this is wrong. Introvert, as an appendix, does not have posterior end, because the "posterior region" is not an end (it continues with the trunk). You have to say "proximal" or "distal" region. Which is this?

Eliminado: color

Eliminado: of

Comentado [ML43]: In descriptions you do not suppose things.

Comentado [ML44]: Telegraphic descriptions do not use verb to be. Re-write the sentence.

Comentado [ML45]: (Fig. 2A,F,G)

Comentado [ML46]: it must be one or the other, but two terms is not used. You have to use the standardized way to name structures. If there is other terms, you have to discuss this in the section, not here.

Comentado [ML47]: confuse: 90° with perpendicular (45°)? Not understandable.

Eliminado: never

Con formato: Subrayado

Eliminado: a

Comentado [ML48]: Main tooth? Distal tip? confuse

Comentado [ML49]: I could say this in proportions.

Comentado [ML50]: All this paragraph needs to be re_q1

Comentado [ML51]: Superlative always has "the": the_q2

Eliminado: a

Con formato: Fuente: Negrita

Comentado [ML52]: Not in telegraphic style

Eliminado: median

Comentado [ML53]: Since the description is made ... [3]

Comentado [ML54]: Including Antilles, or as you say [4]

357

358 **Remarks.** Keferstein (1865) described *Phascolosoma* (*Phascolosoma*) *varians* from St. Thomas,
 359 West Indies and *P. (P.) nigrescens* from Fiji. Although his descriptions were well illustrated,
 360 Selenka (1883) redrew better the type materials (Fig. 3). Keferstein (1865) recognized both
 361 species as follow: *Phascolosoma varians* with a body three to four times as long as thick;
 362 introvert as long or longer than the body; closely spaced rows of hooks, very variable in numbers
 363 (12-90), which often only cover the foremost part of the trunk; hooks very broad, with an upper
 364 right-angled tip (0.072 mm high, 0.092 mm wide); with 20-28 short tentacles, standing in two
 365 rows at the side; musculature strong, about thirty longitudinal muscles, but in many cases
 366 anastomosed as longitudinal strands; contractile vessel on the esophagus simple; nephridia very
 367 long, attached in the anterior third by a mesentery. Whereas *Phascolosoma nigrescens* has a
 368 trunk about four times as long as thick; introvert longer than the trunk; numerous hooks narrowly
 369 spaced, forming rings situated very close to each other in the anterior end of the trunk; hooks
 370 flattened with a small curved hooked tip (0.084 mm high, 0.084 mm wide); over twenty tentacles
 371 in several rows; muscles strong, separated in about 24 longitudinal strands with few anastomose
 372 onces; contractile vessel on the esophagus with many small, lateral sags; nephridia attached
 373 almost to their entire length by a wide mesentery.

374 Cutler & Cutler (1990) reviewed the subgenus *Phascolosoma* (*Phascolosoma*), and
 375 included *P. (P.) varians*, together with other 14 species from different regions of the world, as a
 376 junior synonym of *P. (P.) nigrescens*. Their decision was based on this premise "One possible
 377 hypothesis is that the morphology is determined by more than one pair of genes and that allelic
 378 frequencies vary from place to place. The alleles for sharp angle and large secondary tooth
 379 occur at a high frequency in the Caribbean and a low frequency in the Indo-West Pacif.
 380 *Phascolosoma (P.) varians* is the junior name because it was described later on the page". Later,
 381 Cutler (1994) ratified this synonymy, considering that the morphological differences between *P.*
 382 (*P.*) *nigrescens* and *P. (P.) varians* are not enough to recognize them as different species.

383 Thus, after the redescription of *P. (P.) varians* herein presented, morphological
 384 differences to reject its senior synonym were found. The most important features to recognize
 385 both species are based on the shape of hooks, the attachment of nephridia to the body wall, and
 386 the contractile vessel on the esophagus. *Phascolosoma (P.) varians* has hooks with a rounded
 387 secondary tooth; the base of the hook is broader than high; most of the anterior hooks (Fig. 2A
 388 and 2F) with a distal tip (main tooth) at 90° angle with respect to the perpendicular line of the
 389 hook; contractile vessel simple; nephridia attached to body wall in the anterior third; whereas *P.*
 390 (*P.*) *nigrescens* has hooks with a quadrate secondary tooth; the base of hook is as broad as high;
 391 most anterior hooks with a distal tip with less of 90° angle with respect to the perpendicular line
 392 of the hook; contractile vessel on the esophagus with many small, lateral sags; nephridia attached
 393 almost to their entire length.

394 The wavy contour of nuchal organ and the specific attachment of the retractor muscles
 395 were not described by Keferstein; thus, these characters are included in this discussion,
 396 Keferstein refers to "tentacles in two rows or many rows, maybe it's about the wavy contour of

Comentado [UdMO55]: Improved the drawings?

Eliminado: e

Comentado [ML56]: Narrow?

Comentado [ML57]: How much is very?

Comentado [ML58]: Is this seen in the additional material or in the Kaferstein material?

Comentado [ML59]: Arranged

Comentado [ML60]: Well-developed

Eliminado: about thirty

Comentado [ML61]: Is this meaning "without many small, lateral sags"? The contrary of the next description? Write both descriptions in comparable style.

Comentado [ML62]: How much is very?

Eliminado: ,

Comentado [ML63]: In the previous species you say body. Is it the same? You have to write both with the same terminology and order of information, in order to be comparable.

Eliminado: closely

Comentado [ML64]: Is this the same as closely or narrowly? If yes, do not repeat information.

Comentado [ML65]: In a right-angle too? Or how? Comparable information.

Comentado [ML66]: Well-developed

Comentado [ML67]: There are more than one premise!

Comentado [ML68]: If you put this between quotation marks you to not have to put it in italics too. Just one thing for distinguish their words. Suggest just quotations.

Eliminado: d

Eliminado: Herein, a

Eliminado: ,

Comentado [ML69]: It is presented morphological differences between these two species. But what about the other species that author included in the tree? You have to be more convincent telling reader that these characters have highly taxonomic importance and stability, explaining them how are in other species. How are these characters in, at least, the most close species?

Eliminado: concerning

Comentado [ML70]: Square?

Comentado [ML71]: For both species? clarify

Eliminado: nevertheless

Eliminado: features

Eliminado: now

Eliminado: described

Comentado [ML72]: Not to use contraction style.

nuchal organ that was observed in this material. The differences between the number of tentacles is not useful to separate both species because it is variable regarding the development stage of the specimen, and the variation is overlapping in both species. Because of the difficulty to establish the exact number of longitudinal muscle bands, it should be considered cautiously in recognizing species. Also, the number of hook rings is variable between both species, being the loss of rings very common. Number of papillae seems to be a character without differences between both species.

Molecular analyses

The eleven analyzed sequences revealed six distinct groups of *Phascolosoma (P.) nigrescens* (Fig. 4). The genetic distance between the sequences of *Phascolosoma (P.) nigrescens* from New Caledonia (mean distance 18%) is high. In comparison, the intraspecific variation of *P. (P.) varians* is 2.6% and the mean distance between the sequences of *Phascolosoma (P.) agassizii* from the Pacific coast of USA is 1.0%. Considering that New Caledonia is the closest locality to Fiji — the type locality of *P. (P.) nigrescens* —, it is probable these two sequences correspond to different morphotypes and either one of them can actually correspond with *Phascolosoma (P.) nigrescens*.

The reinstated species group, *Phascolosoma (P.) varians* — from the Greater Caribbean — is clearly separated from the other groups, mainly from the Pacific specimens. Even, this analysis shows that the species *Phascolosoma (P.) varians* is closer to *Phascolosoma (P.) granulatum* than the *P. (P.) nigrescens* from Broome, Australia. The value of the genetic distance between *P. (P.) nigrescens* from Broome, Australia and *Phascolosoma (P.) varians* is 18%. This value is similar to the results of Silva-Morales *et al.* (2019); they found a genetic distance of 19% between *Antillesoma antillarum* from the Greater Caribbean and *Antillesoma mexicanum* from the Southern Mexican Pacific. This mean that *Phascolosoma (P.) varians* from the Greater Caribbean is well differentiating morphologically and genetically of *Phascolosoma (P.) nigrescens* from Broome, Australia.

Discussion

The genetic analysis suggests the following considerations: 1) the specimens from Israel, South Africa and Broome, Australia identified as *Phascolosoma (P.) nigrescens* would corresponds to different species. It is very likely that other species from those regions, considered synonyms, need to be reinstated or some new species be described. 2) It is necessary to clarify the species complex of *Phascolosoma (P.) nigrescens* combining molecular and morphological data. Both suggestions are not resolved in this work because is beyond the scope of this study.

Studies about the distribution of sipunculans have rejected the supposed wide distribution of some species. However, in none of those studies, a new species had been described, or a name reinstatement had been made. For example, Staton & Rice (1999) found a genetic separation

Eliminado: I

Eliminado: s

Eliminado: between

Eliminado: of

Eliminado: ing

Eliminado: s

Eliminado: and

Eliminado: is

Eliminado: P

Eliminado: is an inappropriate character to difference both species because there are

Eliminado: no

Comentado [ML73]: I see molecular analysis not clear, not well explained. It is not easy no follow the explanations with the tree. Recommend to reconsider if it is needed this molecular analysis since it does not explaining in a proper way, the reinstatement of the species. There is not clear evidence with sequences, with distances from other material, more that few numbers given in the two paragraphs. Author(s) must think if not taking into account the molecular analysis, the results are the same. Better than to improve the molecular discussion, is to improve the morphologica discussion.

Comentado [ML74]: I do not know if I am understanding the tree, but the author are naming 7 groups, with al l the species. From them, only 4 have *P. (P.) nigrescens*.

Comentado [ML75]: Not clear. These two groups are the most distant clades. So, why to talk about them? →

Comentado [ML76]: ???

Comentado [ML77]: This is risky. You have to have sequences from Fiji to be sure, otherwise to say "probable" is not the correct way.

Comentado [ML78]: ???

Comentado [ML79]: But the Pacific specimens are very far in the tree. This is sure, also because this Pacific is the northern Pacific, not even the Tropical Pacific.

Comentado [ML80]: Wasn't this the sister group (for you, the comparison group)? So, was it a good group [6]

Comentado [ML81]: This paragraph presents conclusions, that could be at the end of discussion.

Comentado [ML82]: Different species from *P. (P.) nigrescens* or different species between them? Be more

Comentado [ML83]: How much? The word "very" is very imprecise!!!

Comentado [ML84]: This is the first time this concept appears in the document. Why it was not included in the

Comentado [ML85]: Grammar

461 between the genotypes of *Apionsoma* (A.) *misakianum* (Ikeda, 1904) from the northern and
462 southern of Florida using allozymes, even they did not find an indication of “hybrids” occurring
463 between them, establishing the presence of a species-complex, they did not carry out a
464 morphological analysis.

465 Kawauchi & Giribet (2010) rejected the cosmopolitanism of *Phascolosoma* (P.)
466 *perlucens* Baird, 1868, analyzing molecular and morphological data of specimens from many
467 localities around the world. They found four different lineages and identified variation in hook
468 morphology in some localities correlates with a high genetic diversity between populations.
469 Also, their results suggest a probable lack of gene flux between the lineages. Schulze *et al.*
470 (2012) analyzed the molecular data and developmental features of three “cosmopolitan” species,
471 *Phascolosoma* (P.) *agassizii* Keferstein, 1866, *Thysanocardia nigra* (Ikeda, 1904), and *Themiste*
472 (*T.*) *pyroides* (Chamberlin, 1920). They found significant differences between the populations
473 from the Sea of Japan and the Northeast Pacific region, with respect to egg size, developmental
474 mode and developmental timing. The populations of all three species were remarkably distinct
475 genetically and the gene flow between the two regions was extremely unlikely. Also, Kawauchi
476 & Giribet (2014) analyzed *Sipunculus* (S.) *nudus* Linnaeus, 1766 with specimens from many
477 localities worldwide; they found high levels of genetic differentiation between distantly related
478 populations, identifying five distinct lineages, from which three could be distinguished
479 morphologically. In the last two studies, neither new species was described, or an available name
480 was reinstated.

482 **Conclusions**

483 The high genetic divergence between the specimens from the Greater Caribbean regarding those
484 specimens identified as *P. (P.) nigrescens* from a region close to its type locality, support the
485 morphological differences found between *P. (P.) varians* and *P. (P.) nigrescens*. Herein, the
486 synonymy of *Phascolosoma* (P.) *variens* with *Phascolosoma* (P.) *nigrescens* is rejected; as a
487 consequence, *Phascolosoma* (P.) *variens* is reinstated.

488 Ultimately, the diversity of sipunculans is underestimated. Combining morphological and
489 molecular data or other information, as ecological features, will allow us to know the real
490 number of sipunculans species.

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499 the translation.

500

Eliminado: ;

Eliminado:

Eliminado: but

Comentado [ML86]: I tried to improve the sentence, but it needs grammar revision.

Comentado [ML87]: Cosmopolitism?

Eliminado: y

Comentado [ML88]: Grammar

Comentado [ML89]: Ufff... depending on the sequences used. But if it was made with DNA, it not easy to come to this conclusion.

Eliminado: and

Eliminado: were identified

Eliminado: three of

Eliminado: ment

Comentado [ML90]: The real sipunculan diversity.

Comentado [ML91]: Sadly, she is gone! You have to indicate it.

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Página 8: [1] Comentado [ML50] Mario Londoño 31/8/20 23:17:00

All this paragraph needs to be re-write in telegraphic style and more clear. Using Cutler terminology you have to realize that a basic scheme is needed to explain this better. Otherwise, this "rare" terminology makes the paragraph not clear.

Página 8: [2] Comentado [ML51] Mario Londoño 31/8/20 23:15:00

Superlative always has "the": the smallest, the largest, ...

Página 8: [3] Comentado [ML53] Mario Londoño 31/8/20 23:22:00

Since the description is made using only one specimen (UF 332) range in nombre is imposible. How many bands does the 332 have exactly?

Página 8: [4] Comentado [ML54] Mario Londoño 31/8/20 21:53:00

Including Antilles, or as you say West Indies. Remember that the Greater Caribbean is not only the continental shelf.. it includes also all the Greater and Lesser Antilles

Página 9: [5] Comentado [ML69] Mario Londoño 1/9/20 21:55:00

It is presented morphological differences between these two species. But what about the other species that author included in the tree? You have to be more convincent telling reader that these characters have highly taxonomic importance and stability, explaining them how are in other species. How are these characters in, at least, the most close species (phylogenetically, geographically or morphologically)? I think the reinstatement is still weak in arguments.

Página 10: [6] Comentado [ML80] Mario Londoño 1/9/20 23:00:00

Wasn't this the sister group (for you, the comparison group)? So, was it a good group for this? You have to discuse also the behavoiur of the sister/out groups. The tree does not show a relevant outgroup

Página 10: [7] Comentado [ML82] Mario Londoño 1/9/20 21:57:00

Different species from P. (P.) nigrescens or different species between them? Be more precise.

Página 10: [8] Comentado [ML84] Mario Londoño 1/9/20 22:02:00

This is the first time this concept appears in the document. Why it was not included in the introduction, objectives, etc.? I consider it as very important to include in all document in order to justify the molecular analysis, form example. Who else is considering this species a species-complex? Why? Etc.